Claim list:

1. **(Withdrawn)** A multi-layer passive microfluidic mixing device comprising:

a first microfluidic channel defined through the entire thickness of a first stencil layer

a second microfluidic channel defined through the entire thickness of a second stencil layer; and

an overlap region that promotes mixing between at least two different fluid streams, the overlap region being in fluid communication with both the first channel and the second channel.

2 **(Withdrawn)** The mixing device of claim 1 wherein the first stencil layer is adjacent to and in spacerless contact with the second stencil layer.

3. **(Withdrawn)** The mixing device of claim 2, further comprising a spacer layer disposed between the first stencil layer and the second stencil layer, the spacer layer defining an aperture for communicating fluid between the first channel and the second channel.

4. **(Withdrawn)** The mixing device of claim 3 wherein the first channel has a characteristic width, the second channel has a characteristic width, and aperture is at least as large in major dimension than the first channel width or the second channel width.

5. **(Withdrawn)** The mixing device of claim 3 wherein the first channel has a characteristic width, the second channel has a characteristic width, and the aperture is substantially smaller in major dimension than the first channel width or the second channel width.

6. **(Withdrawn)** The mixing device of claim 4 wherein the aperture is disposed substantially centered along the width of the first channel and along the width of the second channel.

7. **(Withdrawn)** The mixing device of claim 3 wherein the first channel has a characteristic width, the second channel has a characteristic width, and the spacer layer

defines a plurality of apertures each substantially smaller in major dimension than the first channel width or the second channel width.

8. **(Withdrawn)** The mixing device of claim 3 wherein the aperture is configured in the shape of a slit.

9. **(Withdrawn)** The mixing device of claim 1 wherein the overlap region has a characteristic width, and, within or immediately adjacent to the overlap region, contact between the at least two different fluid streams is established along the entire width of the overlap region.

10. **(Withdrawn)** The mixing device of claim 1 wherein both the first channel and the second channel are located substantially upstream of the overlap region.

11. **(Withdrawn)** The mixing device of claim 1 wherein the first channel supplies a first fluid stream to the overlap region and the second channel supplies a second fluid stream to the overlap region.

12. **(Withdrawn)** The mixing device of claim 1 wherein the first channel supplies a stream of a first fluid to the overlap region and the second channel receives a stream of multiple fluids from the overlap region.

13. **(Withdrawn)** The device of claim 1 wherein the first channel supplies a stream of multiple fluids to the overlap region and the second channel receives a stream of multiple fluids from the overlap region.

14. **(Withdrawn)** The device of claim 13 wherein the first channel includes a junction for merging a first fluid stream with a second fluid stream.

15. **(Withdrawn)** The device of claim 1 wherein the first channel is located substantially upstream of the overlap region and the second channel is located substantially downstream of the overlap region.

16. **(Withdrawn)** The device of claim 1 wherein the first channel is located substantially upstream of the overlap region and the second channel includes an upstream segment located substantially upstream of the overlap region and a downstream segment located substantially downstream of the overlap region, the upstream segment and the downstream segment being substantially continuous.

17. **(Withdrawn)** The device of claim 16 wherein the upstream segment has a characteristic width, the downstream segment has a characteristic width, and the width of the downstream segment is greater than the width of the upstream segment.

18. **(Withdrawn)** The device of claim 1 wherein the overlap region includes a third channel defined through the entire thickness of a third stencil layer disposed between the first stencil layer and the second stencil layer.

19. **(Withdrawn)** The device of claim 1 wherein, immediately upstream of the overlap region, a first fluid stream is directed in a first direction and, separately from the first fluid stream, a second fluid stream is directed in substantially the same direction.

20. **(Withdrawn)** The device of claim 1 wherein upstream of the overlap region a combined stream of multiple fluids travels in a first direction, and immediately downstream of the overlap region the combined stream of multiple fluids is manipulated to undergo a substantial change in direction relative to the first direction.

21. **(Withdrawn)** The device of claim 21 wherein the directional change is at least about 90 degrees.

22. **(Withdrawn)** The device of claim 1, further comprising a first fluid inlet port and a second fluid inlet port.

23. **(Withdrawn)** The device of claim 22, further comprising a third fluid inlet port and a third channel defined through the entire thickness of a third stencil layer, wherein mixing is promoted between more than two fluid streams.

24. **(Withdrawn)** The device of claim 1, further comprising an outlet port.

25. **(Withdrawn)** The device of claim 1 wherein mixing is promoted by at least two fluid streams in a plurality of different proportions.

26. **(Withdrawn)** The mixing device of claim 1 wherein the device is constructed with multiple layers, and the various layers are bonded or fastened together.

27. **(Withdrawn)** The device of claim 26 wherein the bonded or fastened layers form a substantially sealed device.

28. **(Withdrawn)** A multi-layer microfluidic mixing device comprising:

a first microfluidic channel for transporting a first fluid stream, the first channel being defined through the entire thickness of a first stencil layer;

a second microfluidic channel for transporting a second fluid stream, the second channel being defined through the entire thickness of a second stencil layer;

a microfluidic outlet channel; and

an overlap region for contacting the first fluid stream with the second fluid stream in the outlet channel to promote mixing between the fluid streams.

29. **(Withdrawn)** The mixing device of claim 28 wherein the outlet channel has a characteristic height and a characteristic width, and contact between the first fluid stream and the second fluid stream is established in the outlet channel along the entire width of the outlet channel.

30. **(Withdrawn)** The mixing device of claim 28 wherein the outlet channel has a characteristic height and a characteristic width, and contact between the first fluid stream and the second fluid stream is established in the outlet channel along an interfacial area that is as least as large per unit length as the greater of the height or the width of the outlet channel.

31. **(Withdrawn)** The mixing device of claim 28 wherein, immediately upstream of the overlap region, the first microfluidic channel directs the first fluid stream in a first direction,

and the second microfluidic channel directs the second fluid stream in substantially the same direction.

32. **(Withdrawn)** The mixing device of claim 28 wherein the outlet channel is defined through the entire thickness of a third stencil layer, the third stencil layer being disposed between and in spacerless contact with both the first stencil layer and the second stencil layer.

33. **(Withdrawn)** The mixing device of claim 28, the device further comprising a spacer layer disposed between the first stencil layer and the second stencil layer, the spacer layer defining an aperture that permits fluid communication between the first fluid stream and the second fluid stream.

34. **(Withdrawn)** The mixing device of claim 33 wherein the first channel has a characteristic width, the second channel has a characteristic width, and aperture is at least as large in major dimension than the width of the first channel or of the second channel.

35. **(Withdrawn)** The mixing device of claim 34, wherein the aperture is configured in the shape of a slit.

36. **(Withdrawn)** The mixing device of claim 35, wherein the slit is disposed substantially perpendicular to the direction of bulk fluid flow in first and the second microfluidic channel.

37. **(Withdrawn)** The mixing device of claim 33, wherein the spacer layer defines a plurality of apertures

38. **(Withdrawn)** The mixing device of claim 37 wherein the outlet channel has a nominal width, and each aperture is substantially smaller in major dimension than the width of the outlet channel.

39. **(Withdrawn)** The mixing device of claim 28, wherein the outlet channel is defined through the entire thickness of the second stencil layer and is a substantially continuous extension of the second microfluidic channel.

40. **(Withdrawn)** The mixing device of claim 28 wherein:

the second channel is a channel segment upstream of the overlap region;

the outlet channel is a channel segment defined through the entire thickness of the second stencil layer and located downstream of the overlap region; and

the second channel and the outlet channel are substantially continuous.

41. **(Withdrawn)** The mixing device of claim 40 wherein the second channel has a characteristic width, the outlet channel has a characteristic width, and the width of the outlet channel is larger than the width of the second channel.

42. **(Withdrawn)** The mixing device of claim 28 wherein both the first channel and the second channel are located substantially upstream of the overlap region, and the outlet channel is located substantially downstream of the overlap region.

43. **(Withdrawn)** The mixing device of claim 28 further comprising a third microfluidic channel for transporting a third fluid stream, the third channel being defined through the entire thickness of a third stencil layer, wherein the device promotes mixing between the first fluid stream, second fluid stream, and third fluid stream.

44. **(Withdrawn)** The mixing device of claim 28, further comprising at least two fluid inlet ports and a plurality of fluid outlet ports.

45. **(Withdrawn)** The mixing device of claim 28 wherein the device is constructed with multiple layers, and the various layers are bonded or fastened together.

46. **(Withdrawn)** The device of claim 45 wherein the bonded or fastened layers form a substantially sealed device.

47. **(Original)** A microfluidic device for mixing a plurality of fluid streams, the mixing device comprising:

a plurality of microfluidic inlet channels that merge into a microfluidic junction channel, the junction channel being defined in a first device layer and having a characteristic cross-sectional area; and

a plurality of contraction / expansion regions in fluid communication with the junction channel and arranged in series, each contraction / expansion region including:

an aperture defined in a second device layer, the aperture having a characteristic cross-sectional area that is substantially smaller than the area of the junction channel; and

a microfluidic expansion channel defined in either the first device layer or a third device layer, the expansion channel having a characteristic cross-sectional area that is substantially larger than the area of the aperture.

48. **(Original)** The mixing device of claim 47 wherein each aperture is less than about 250 microns in major dimension.

49. **(Original)** The mixing device of claim 48 wherein:

the junction channel contains a stream of multiple fluids;

upstream of an aperture, the stream of multiple fluids flows in substantially a first direction;

downstream of an aperture, the stream of multiple fluids flows in substantially a second direction that is substantially different from the first direction.

50. **(Original)** The mixing device of claim 49 wherein the second direction is at least about 90 degrees apart from the first direction.

51. **(Original)** The mixing device of claim 47 wherein any of the inlet channels, junction channel, or expansion channels are defined through the entire thickness of a stencil layer.

52. **(Original)** The mixing device of claim 47 wherein any of the inlet channels, junction channel, or expansion channels are defined in a surface but do not penetrate the entire thickness of a device layer.

53. **(Original)** The mixing device of claim 52 wherein any of the inlet channels, junction channel, or expansion channels are defined using one or more surface micromachining techniques.

54. **(Original)** The mixing device of claim 47 wherein the device is formed with multiple layers, and the various layers are bonded or fastened together.

55. **(Original)** The mixing device of claim 54 wherein the bonded or fastened layers form a substantially sealed device.

56. **(Currently amended)** A multi-layer microfluidic mixing device comprising:

a first device layer, a third device layer, and a second device layer disposed between the first device layer and the third device layer;

a plurality of microfluidic inlet channels that merge into a junction channel, the junction channel being defined in [[a]] the first device layer and having a characteristic width;

a slit defined in [[a]] the second device layer, the slit having a characteristic length and width and being ~~in fluid communication with the junction channel and~~ disposed lengthwise in a direction substantially parallel to the junction channel, the slit length being substantially greater than the slit width; and

a microfluidic outlet channel defined in [[a]] the third device layer and having a characteristic width , the outlet channel being ~~in fluid communication with the slit~~ disposed in a direction substantially perpendicular to both the junction channel and the slit;

wherein the slit is disposed between and in fluid communication with the junction channel and the outlet channel, the slit width is substantially smaller than the junction channel width, and the slit width is substantially smaller than the outlet channel width.

57. **(Canceled)**

58. **(Currently amended)** The mixing device of claim 56 wherein ~~the slit has a characteristic length, the outlet channel has a characteristic width, the slit has a characteristic length, and the length of~~ the slit length is at least as great as ~~the width of~~ the outlet channel width.

59. **(Currently amended)** The mixing device of claim 56 wherein ~~any of the inlet channels, junction channel, or outlet channel are defined through the entire thickness of a stencil layer~~ the first device layer is a first stencil layer, the third device layer is a third stencil layer, the junction channel is defined through the entire thickness of the first stencil layer, and the outlet channel is defined through the entire thickness of the third stencil layer.

60. **(Currently amended)** The mixing device of claim 56 wherein ~~any of the inlet channels,~~ the junction channel~~, or outlet channel are~~ is defined in a surface ~~but do~~ of but does not penetrate the entire thickness of [[a]] the first device layer and the outlet channel is defined in a surface of but does not penetrate the entire thickness of the third device layer.

61. **(Currently amended)** The mixing device of claim 56 wherein any of the plurality of inlet channels, the junction channel, or the outlet channel [[are]] is defined using one or more surface micromachining techniques.

62. **(Original)** The mixing device of claim 56 wherein the junction channel is substantially upstream of the slit, and the outlet channel is substantially downstream of the slit.

63. **(Currently amended)** The mixing device of claim 56 wherein ~~the device is formed with multiple layers, and the various layers~~ first device layer, second device layer, and third device layer are bonded or fastened together to form a substantially sealed device.

64. **(Canceled)**

65. **(Canceled)**

66. **(Canceled)**

67. **(Canceled)**

68. **(Canceled)**

69. **(Canceled)**

70. **(Canceled)**

71. **(Canceled)**

72. **(Canceled)**

73. **(Canceled)**

74. **(Currently amended)** A microfluidic mixing device comprising:

a first device layer, a second device layer, and a third device layer disposed between the first device layer and the second device layer;

a first microfluidic channel defined in [[a]] the first device layer, the first channel having a characteristic width;

a second microfluidic channel defined in [[a]] the second device layer, the second channel having a characteristic width ; and

a plurality of apertures defined in the third device layer, the plurality of apertures being disposed between and in fluid communication with the first channel and the second channel, ~~the apertures being defined in a third device layer disposed between the first layer and the second layer~~ each aperture of the plurality of apertures having a major dimension that is substantially smaller than each of the width of the first channel and the width of the second channel.

75. **(Currently amended)** The mixing device of claim 74 wherein ~~the first channel has a characteristic width, the second channel has a characteristic width, and each aperture has a major dimension that is substantially smaller than~~ the width of the first channel is substantially equal to [[or]] the width of the second channel.

76. **(Currently amended)** The mixing device of claim 75 wherein the major dimension of each aperture of the plurality of apertures is less than about one-quarter of each of the width of the first channel [[or]] and the width of the second channel.

77. **(Original)** The mixing device of claim 75 wherein each aperture has a major dimension of less than about 200 microns.

78. **(Original)** The mixing device of claim 75 wherein each aperture has a major dimension of less than about 100 microns.

79. **(Currently amended)** The mixing device of claim 74 wherein the first channel has a characteristic cross-sectional area, the second channel has a characteristic cross-sectional area, each aperture has a characteristic cross-sectional area, and the area of each aperture [[us]] is substantially smaller than the area of the first channel and the area of the second channel.

80. **(Original)** The mixing device of claim 74 wherein the first channel is substantially upstream of the plurality of apertures and the second channel is substantially downstream of the plurality of apertures.

81. **(Currently amended)** The mixing device of claim 74 wherein the first device layer is a first stencil layer with the first channel being defined through the entire thickness of the first stencil layer and the second device layer is a second stencil layer with the second channel being ~~any of the first channel or the second channel are~~ defined through the entire thickness of [[a]] the second stencil layer.

82. **(Currently amended)** The mixing device of claim 74 wherein: ~~any of~~ the first channel ~~or the second channel are~~ is defined in a surface of [[a]] the first device layer but [[do]] does not penetrate the entire thickness of the first device layer; and

the second channel is defined in a surface of the second device layer but does not penetrate the entire thickness of the second device layer.

83. **(Original)** The mixing device of claim 82 wherein any of the first channel or the second channel are defined using one or more surface micromachining techniques.

84. **(Currently amended)** The mixing device of claim 74 wherein the first device layer, second device layer, and third device layer ~~device is formed with multiple layers, and the various layers~~ are bonded or fastened together.

85. **(Currently amended)** The mixing device of claim 74, further comprising a fourth device layer and a fifth device layer, wherein the first through fifth device layers are bonded or fastened ~~layers~~ together to form a substantially sealed device.

86. **(Withdrawn)** A microfluidic mixing device for mixing different fluids in multiple proportions, the device comprising:

a first microfluidic channel having a forked region for splitting a first fluid stream into multiple sub-streams;

a second microfluidic channel having a forked region for splitting a second fluid stream into multiple sub-streams;

a plurality of overlap regions each contacting a sub-stream of the first fluid with a sub-stream of the second fluid to promote mixing between the fluid sub-streams.

87. **(Withdrawn)** The mixing device of claim 86 wherein the device produced multiple mixed output streams each having a different proportion of the first fluid and/or the second fluid.